Evaluation of Clindamycin encapsulated in PLA/PLGA nanoparticles

Presented by
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My area of Research

My Background: I have mostly worked on pharmacological and immunological aspects of various polymer formulations encapsulating various antigens as vaccine candidates.

Here in NIT, I am working on drug/vaccine delivery systems, biomaterials, tissue engineering, scaffolds and their immunological aspects, probiotics etc. The various areas are:

1. Drug Delivery- Clindamycin, OMP/OMV antigens like Aeromonas/vibrio
2. Tissue Engineering- Cryopreservation by various cryoprotectants
3. Nanoparticle mediated biomaterials and their interactions with other biomolecules
4. Probiotics
**Introduction**

- In our body various acidic and alkaline medium are present so when drugs passes through these medium its physical and chemical properties gets changed- No optimum immune response.

- By the time it reach its target site, it may be degraded or its effectiveness may be shunted or less.

- In conjugation into PLA/PLGA nanoparticle, we have tried to make the drug loaded particles less susceptible to various acidic medium in our body and increasing its efficiency and Immune response.
10 Leading killers in the world of infectious diseases. All are through mucosal sites. (Exception: malaria and neonatal tetanus)
Drug: Structure of Clindamycin hydrochloride
Clindamycin hydrochloride

- Clindamycin Hydrochloride which is the hydrochloride salt of clindamycin it is white crystalline powder in nature and administered orally.
- It is used primarily in the treatment of penicillin-resistant infections and in patients allergic to penicillin.
- Serum level studies with a 150 mg oral dose of clindamycin hydrochloride showed that it was rapidly absorbed after oral administration.
• Clindamycin can also be treated in case of serious infections due to susceptible strains of Streptococci, Pneumocooci and Styphalocooci.

• Clindamycin hydrochloride antibiotics is also used for the treatment of infected wounds, abscesses, and dental infections in dogs and cats and osteomyelitis in dogs.

• This drug also be used before dental procedures in patients with certain heart conditions (e.g., artificial heart valves) to help prevent serious infection of the heart (bacterial endocarditis).

• Clindamycin hydrochloride is used to treat a wide variety of bacterial infection. It is an antibiotic that works by stopping the growth of bacteria.
Objectives

• To develop and characterize Clindamycin hydrochloride encapsulated drug which is generally used in various bacterial infection diseases.

• To establish a suitable polymeric oral delivery system for Clindamycin with enhanced bioactivity.
MICROSPHERE ENCAPSULATION

Controlled Delivery – POLYMERS (Vehicles)

Biodegradable Polymers

Biocompatible Polymers

FDA Approved- PLA/PLGA
ANTIGEN / DRUG RELEASE

Encapsulation

Antigen/Drug Release
Biopolymers

- Collagen & gelatin
- Chitin & chitosan
- Alginate
- Cellulose derivatives
- Starch
- Carrageenan
- Agar
- Pectin

- Dextran
- Gums → Guar gum, gum acacia, gum tragacanth
  - PLA
  - PGA
  - PLA-co-PGA
PREPARATION OF PLA/PLGA NANOPARTICLE AND CONJUGATION WITH CLINDAMYCIN HYDROCHLORIDE DRUG FOLLOWED BY CHARACTERIZATION STUDIES
Method of Preparation

ORGANIC PHASE
(POLYMER+DCM+ACETONE)

INTERNAL AQUEOUS PHASE
(CLINDAMYCIN HYDROCHLORIDE+PBS)

EMULSIFICATION (SONICATION)

INNER EMULSION
(W₁+O)

WATER+PVA
(W₂)

EMULSIFICATION (SONICATION)

SECONDARY EMULSION
(W₁/O/W₂)
DCM
ACETONE
(CENTRIFUGATION)

NANOPARTICLE AND MICROPARTICLE FORMATION

LYPHOLIZED TO RECOVER THE PARTICLE
PARTICLE SIZE AND ZETA POTENTIAL ANALYSIS

• The lyophilized samples were diluted with PBS of 67 ml and ph 6.0 on mg/ml and analyzed.
• The size, size distribution and zeta potential of the nanoparticles were analyzed by Zeta seizer.

DSC ANALYSIS

The physical state of Clindamycin hydrochloride entrapped in the nanoparticles as well as the blank nanoparticles of PLA and PLGA were characterized by differential scanning calorimetry DSC thermogram analysis.
• Then glass transition temperature were analyzed.
SEM ANALYSIS

• To observe surface morphology of NP, the nanoparticles were fixed on adequate supports and coated with platinum using platinum sputter module in a higher vacuum evaporator.

FTIR ANALYSIS

• On FTIR analysis of PLA /PLGA conjugated Clindamycin hydrochloride sample were done to determine the quality or consistency of a sample

RELEASE PROFILE & ANTIMICROBIAL TEST

• The cumulative release of the drug from the highest encapsulated or drug loaded particles were studied and antimicrobial test was done.
RESULTS
Size & Zeta potential measurements of the particles

Particle size of blank-PLA 42.93 nm
Particle size of clindamycin hydrochloride-PLA 323.5 nm
Zeta potential of blank -PLA -24.8 MV

(SAMPLE-L)
Zeta potential of clindamycin hydrochloride-PLA -11.5 MV

(SAMPLE-2)
Particle size of blank-PLGA 258.3 nm
Zeta size of clindamycin hydrochloride-PLGA 178.6nm

(SAMPLE-3)
Zeta potential of blank -PLGA - -32.7 MV

(SAMPLE-G)
Zeta potential of clindamycin hydrochloride-PLGA -17.5 MV

(SAMPLE-3)
### Size and zeta potential correlation

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Sample Name</th>
<th>Mean Particle Size</th>
<th>Zeta Potential</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>42.93nm</td>
<td>-24.8mv</td>
</tr>
<tr>
<td>2</td>
<td>G</td>
<td>258.3nm</td>
<td>-32.7mv</td>
</tr>
<tr>
<td>3</td>
<td>2</td>
<td>323.5 nm</td>
<td>-11.5 mv</td>
</tr>
<tr>
<td>4</td>
<td>3</td>
<td>178.6nm</td>
<td>-17.5 mv</td>
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</tbody>
</table>
SEM MICROGRAPHS

PLA + CLH NANOPARTICLE

PLGA+CLH NANOPARTICLE
Physical properties of Clindamycin loaded PLA and PLGA nanoparticles

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Sample (Code)</th>
<th>Conc. of Drug (mg)</th>
<th>Conc. of Polymer (mg)</th>
<th>Ratio of drug: Polymer</th>
<th>Mean Particle size (Diameter in nm ± SD)</th>
<th>Zeta potential (MV) ± SD</th>
<th>Poly Dispersity Index (PDI) ± SD</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PLA</td>
<td>Blank (PLA)</td>
<td>-</td>
<td>400</td>
<td>-</td>
<td>42.93 ± 1.77</td>
<td>-24.8 ± 7.67</td>
<td>0.454 ± 0.05</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CLH- PLA 1</td>
<td>20</td>
<td>400</td>
<td>1:20</td>
<td>203.35 ± 12.04</td>
<td>-19.8 ± 3.88</td>
<td>0.332 ± 0.03</td>
<td>7.2 ± 2.08</td>
</tr>
<tr>
<td></td>
<td>CLH- PLA 2</td>
<td>40</td>
<td>400</td>
<td>1:10</td>
<td>323.5 ± 16.39</td>
<td>-30.5 ± 4.95</td>
<td>0.219 ± 0.01</td>
<td>21.35 ± 3.17</td>
</tr>
<tr>
<td></td>
<td>CLH- PLA 3</td>
<td>80</td>
<td>400</td>
<td>1:5</td>
<td>827.4 ± 10.20</td>
<td>-17.6 ± 6.55</td>
<td>0.423 ± 0.04</td>
<td>24.5 ± 4.29</td>
</tr>
<tr>
<td>PLGA</td>
<td>Blank (PLGA)</td>
<td>-</td>
<td>400</td>
<td>-</td>
<td>178.6 ± 12.11</td>
<td>-32.7 ± 5.01</td>
<td>0.524 ± 0.03</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>CLH-PLGA 1</td>
<td>20</td>
<td>400</td>
<td>1:20</td>
<td>196.45 ± 8.78</td>
<td>-25.5 ± 2.88</td>
<td>0.650 ± 0.05</td>
<td>45 ± 3.45</td>
</tr>
<tr>
<td></td>
<td>CLH-PLGA 2</td>
<td>40</td>
<td>400</td>
<td>1:10</td>
<td>258.3 ± 11.23</td>
<td>-33.5 ± 3.0</td>
<td>0.176 ± 0.01</td>
<td>65.69 ± 2.28</td>
</tr>
<tr>
<td></td>
<td>CLH-PLGA 3</td>
<td>80</td>
<td>400</td>
<td>1:5</td>
<td>456.5 ± 12.36</td>
<td>-21.7 ± 5.34</td>
<td>0.353 ± 0.03</td>
<td>72.35 ± 2.31</td>
</tr>
</tbody>
</table>
DSC ANALYSIS
<table>
<thead>
<tr>
<th>MATERIALS</th>
<th>TEMPERATURE (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clindamycin hydrochloride tablet</td>
<td>150</td>
</tr>
<tr>
<td>Blank PLA</td>
<td>50</td>
</tr>
<tr>
<td>Blank PLGA</td>
<td>170</td>
</tr>
<tr>
<td>Clindamycin hydrochloride-PLA</td>
<td>160</td>
</tr>
<tr>
<td>Clindamycin hydrochloride-PLGA</td>
<td>48</td>
</tr>
</tbody>
</table>
CLINDAMYCIN HYDROCHLORIDE TABLET
CLINDAMYCIN HYDROCHLORIDE PLA
CLINDAMYCIN HYDROCHLORIDE
PLGA
FTIR ANALYSIS
CLINDAMYCIN HYDROCHLORIDE+PLA
CLINDAMYCIN HYDROCHLORIDE + PLGA
IN VITRO CLH RELEASE FROM PLA AND PLGA NANOPARTICLES.
Antibacterial activity of Clindamycin hydrochloride conjugated with PLA (CLH-PLA 2), Clindamycin hydrochloride conjugated with PLGA (CLH-PLGA 2) and Clindamycin hydrochloride, in vitro MIC in µg/mL

| Formulations          | MIC (µg/mL) ± Standard deviation |  
|-----------------------|----------------------------------|---
|                       | Streptococcus faecalis           | Bacillus cereus                  |
| Clindamycin hydrochloride | 0.48± 0.01                      | 1.95±0.04                        |
| PLA-CLH 2             | 0.12±0.05                        | 0.97±0.08                        |
| PLGA-CLH 2            | 0.24±0.05                        | 0.48±0.06                        |
TEM MICROGRAPHS
The surface morphology of PLA microparticle (PLAM), B: The surface morphology of PLA nanoparticle (PLAN).
The surface morphology of PLGA microparticle (PLGAM), B: The surface morphology of PLGA nanoparticle (PLGAN).
CONCLUSIONS
During the current investigation, CLH-PLA and CLH-PLGA nanoparticles of various sizes were formulated by varying the drug to polymer ratio from 1:5 to 1:20. The drug to polymer ratio 1:10 were found to be optimal ratio for the formulation to be stable and monodispersed for both CLH loaded PLA and PLGA nanoparticles.

The CLH-PLGA nanoparticles (size: 258.3±11.23 nm, zeta potential: -33.5 ± 3.0 mv, PDI: 0.176 ± 0.01, loading efficiency: 65.69 ± 2.28%) showed more stable physical properties than CLH-PLA nanoparticles (size: 323.5±16.39 nm, zeta potential -30.5 ± 4.95: mv, PDI: 0.332±0.03, loading efficiency: 21.35±3.17%) at 1:10 drug to polymer ratio.

Nanoparticle formulation (both CLH-PLA and CLH-PLGA) showed a significant controlled release profile extended up to 144 h, but PLGA showed considerably slower release profile than from PLA.

The slower drug release profile for PLGA-drug indicates that PLGA-drug can be a better oral delivery system as it can withstand the drug even after 4 hr which is the standard for retaining food in stomach.

The thermal behaviour (DSC) studies of CLH-PLGA nanoparticles suggested that the drug was dispersed at a molecular level within the system.

From FTIR studies it was found that there was not much alteration in general structure of CLH drug after loading. This shows the drug CLH was also not involved in any chemical interactions with the polymers and it was intact throughout.
The antimicrobial activities were enhanced in clindamycin encapsulated PLA and PLGA nanoparticles than the standard free drug as MIC values were decreased when tested against *Streptococcus faecalis* and *Bacillus cereus*.

The encapsulated drug Clindamycin is more effective at lower concentration against pathogenic microorganisms which can be widely applied in number of therapies.
Lab Members

Lab at Work
Thank you for your kind attention
ANIMAL EXPERIMENT – Followed by ELISA

Balb/C

Oral Route

Swiss Webster

ELISA